

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA

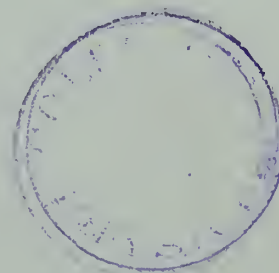
THE DEVELOPMENT OF THE PARASITE OF INDIAN KALA AZAR

BY

CAPTAIN W. S. PATTON, M.B., I.M.S.

Offg. Director, King Institute of Preventive Medicine, Madras.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE
SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA



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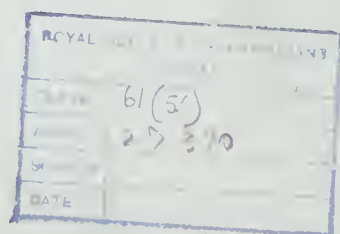
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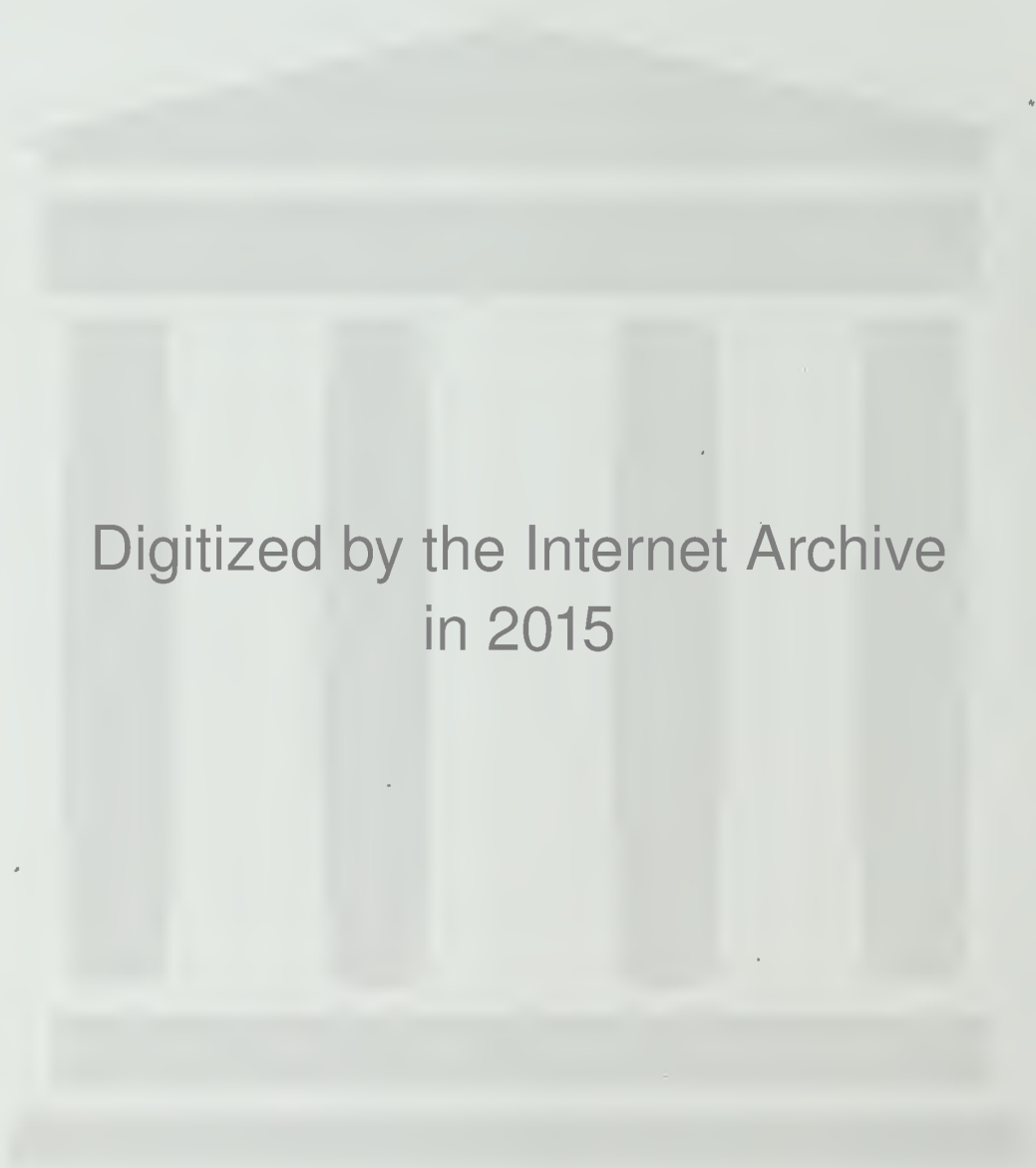


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The Development of the Parasite of Indian Kala Azar (*Herpetomonas Donovanii*. Laveran and Mesnil.) in *Cimex rotundatus*, Sign. and in *Cimex lectularius*, Linn. with some observations on the behaviour of the Parasite in *Conorrhinus rubrofasciatus*, de Geer.

IN 1907 I described, in two memoirs, my experimental work on the etiology of Indian Kala Azar, and I have since then devoted my spare time to the study of the flagellates of the genus *Herpetomonas*, both in blood sucking and non-blood sucking insects, for I was convinced that these studies would enable me to solve the problem of the method of transmission of the human parasite; I hoped some time later to have another opportunity of continuing and completing my work on the etiology of Kala Azar. In January 1912 I was placed on special duty in order to devote my whole time to this work. A suitable case for feeding experiments was soon found in the General Hospital, Madras, and a further discovery has been made, which goes far towards solving the Kala Azar problem. In the present paper I will describe these observations, but before doing so it will not be out of place to review my previous work on this subject, and then to summarize all the recent work on the infantile form of Kala Azar.

In my first memoir on the development of the parasite in *Cimex rotundatus*, I gave a short account of Rogers' discovery of the flagellate stage of the parasite, of Christophers' confirmation of this in Madras, and of the similar work of Leishman and Statham in England. I then discussed the various hypotheses which had been advanced to explain how the human body might be primarily infected by the parasite. Manson and Low's observations on a case of Kala Azar in London led them to conclude that the parasite could not be transmitted by means of the peripheral blood, as they failed to find any parasites in films made from the finger blood, and they suggested that the parasite may escape from the ulcers in the intestinal tract and be passed out in the fæces. Statham, however, refuted this hypothesis for he carried out a series of examinations and cultural experiments with the fæces of a case of Kala Azar, and failed to find the parasite; my observations made along the same lines confirmed Statham's results. Later Manson again advanced the hypothesis that the parasite probably leaves the human body in the discharges from ulcerated surfaces, intestinal or cutaneous, and that it may be ingested by some foul feeding fly in which it may undergo multiplication, and later be deposited when the fly came in contact with broken skin surface.

Critien revives this hypothesis, and in a recent note draws attention to the possibility of the parasite being transmitted by way of the intestinal tract, for he states he has recovered it from the fæces of a case of Kala Azar.

Before it is possible to seriously consider this hypothesis, it is necessary to at least attempt to explain how the parasites, which may be passed out in the human fæces, can again find their way back to a clean person. It is well known that these parasites will not develop into flagellates in any medium infected with bacteria, so that it is difficult to understand how they can ever become flagellates in the human discharges. This being the case, those parasites which are passed out must either be ingested by a fly, or they must immediately be transferred to some broken skin surface on an uninfected person. Now any foul feeding fly such as *Musca nebulo*, Fabr. already contains bacteria in its intestinal tract, and I have carried out a series of experiments by feeding both wild and bred house flies (*Musca nebulo*) on splenic juice obtained from a patient suffering from Kala Azar, and found that the parasites degenerate in a few hours, and could not be recovered in their excrement. It is difficult then to understand how a fly can play any part in the transmission of the disease. If it is true that the parasite is directly transferred to another person, there would then be no necessity for it to flagellate when removed from the human body; it is possible that this may take place in the case of animals. I have, however, no doubt that this method of infection never takes place in the case of man.

The other hypothesis, suggested first by Christophers, and later by Rogers, that the parasite is ingested by a blood-sucking insect, has now been amply supported by feeding experiments. That the parasite may pass into the stomach of a blood-sucking insect it is necessary for it to be present in the circulating blood. Donovan and Christophers, early in their investigation, recorded having found the parasite in the white blood cells in films of finger blood, and I have been able to confirm their observations. In 1906 I found the parasite in 38 out of 45 cases examined in the General Hospital, Madras; I further pointed out that there were certain phases in the disease when the parasites were present in large numbers in the circulating blood. These periods are characterised by the onset of severe dysenteric symptoms pointing to extensive ulceration of the large intestine. It should be noted that these dysenteric attacks may supervene at an early stage of the disease, and the patients may recover from them, a relapse later ending fatally. In many cases it was noted that during these attacks the parasites could be found in large macrophages, and as many as 300 have been found in a single blood film. With regard to the presence of the parasite in the circulating blood, it is necessary to quote a recent statement made by Leishman. Commenting on the method of

demonstrating the presence of the parasite by the examination of the peripheral blood, he says :— “The explanation for the general failure of this method in Kala Azar is two-fold : the parasites, if present at all, are very rare, since they are encountered in cells and not free, the intense leucopenia which is usually found adds greatly to the difficulties of the search. In the Indian form very few positive results have been mentioned, with the striking exception of Donovan’s success at Madras ; as already mentioned, he has demonstrated their presence in the peripheral blood in 93·2 per cent. of his recent Madras cases. On the other hand, Prashad records 213 examinations of finger blood with only one positive result, and the latter’s experience is in accord with that of most observers.” This statement is not a fair comment on the work which has been done in India on this part of the subject, for no mention is made of Christophers’ observations or those of the writer noted above. Since my earlier observations I have only once failed to find the parasite in the blood of an undoubted case of Kala Azar and then it was subsequently found by Donovan. This work requires experience, and no weight can be attached to Prashad’s failures until it is known whether he has had special training in this work. To one who is accustomed to finding the parasite in practically every case, statements such as those quoted above are of no value. In the General Hospital, Madras, the parasite has now been found in a large number of cases at every stage of the disease, and the students are taught to diagnose each case by finding the parasite in films of finger blood ; if this can be done by students, it can surely be accomplished by experienced observers. In a recent paper Cochran states that as he has failed to find the parasite in the peripheral blood in cases of Kala Azar in Anhwei China, he has resorted to the excision of lymph glands, from which smears are made in which the parasite can be readily demonstrated. He states that the examination of these films may occupy from five to twenty minutes. It may be of interest to this observer to know that the writer is able to look over a blood film with a No. 6, 7 or 8 dry objective in much less than five minutes, and can recognise the parasite without using an oil immersion lens. I would strongly recommend this observer to accustom himself to find the parasite with a dry objective in a film containing a large number, such as a smear of splenic juice or that of a lymph gland. The dangerous operation of splenic puncture for diagnostic purposes has long since been given up in Madras, and I feel sure that gland excision would never be tolerated in this country ; both operations are unnecessary and unjustifiable.

The presence of the parasite in large numbers in the circulating blood during dysenteric attacks is of peculiar interest, for Bentley long ago pointed out that during the height of an epidemic in Assam, cases with dysentery

were soon followed by fresh ones in the same house, or among the attendants. I have frequently found in Madras that a second case has occurred in the same house in which a patient died as the result of severe dysentery, and I have recorded two instances of a number of cases in the same house, several of which had severe dysentery which proved fatal.

Experiments with Blood-sucking Insects.

In 1907 I recorded the following feeding experiments, and as these appear to have been lost sight of, it is well to again draw attention to them.

EXPERIMENT WITH LICE, *PEDICULUS CAPITIS*.

As the result of the examination of 149 lice, I came to the conclusion that this insect could not possibly be the natural carrier for I was unable to find the parasite undergoing any developmental changes in its alimentary tract.

PEDICULUS VESTIMENTI.

About fifteen lice were dissected after having fed on a suitable case, but I was unable to find any changes towards development. It is interesting to note that Fantham has recently described the life history of a species of herpetomonas (*H. vestimenti*, *Fantham*) from the alimentary tract of this louse. It is hoped that workers in other parts of the world, who undertake experiments with this louse will carefully keep in view this harmless flagellate.

EXPERIMENTS WITH MOSQUITOES. *CULEX FATIGANS*.

One hundred and fifty-seven female mosquitoes were fed on suitable cases and dissected at varying intervals with negative results. In Madras this mosquito is naturally infected with a herpetomonas (*H. culicis* *Novy*), and it is important to note that the infection is acquired by the larvæ. A complete account of this flagellate will shortly be published.

NEOCELLIA STEPHENSI.

Forty specimens of this anopheles were fed on a case in whose peripheral blood there were many parasites, and they were dissected with negative results. This mosquito is the common anopheles of George Town, one of the endemic foci of Kala Azar, and it was found to be infected naturally with a flagellate which was also present in the larvæ. Reference will be made to this parasite in connection with Franchini's recent experiments.

STEGOMYIA SUGENS.

Thirty-one female mosquitoes, fed several times on a suitable case, were dissected at regular intervals but with negative results; this mosquito does not as far as I know contain a natural flagellate.

EXPERIMENTS WITH TICKS. ORNITHODORUS SAVIGNYI.

Several adults and nymphs were repeatedly fed on a case of Kala Azar shewing parasites in the peripheral blood, and were dissected with negative results.

EXPERIMENTS WITH CIMEX ROTUNDATUS.

Before describing these experiments, it is again necessary to state that this bug never contains, as far as I know, a natural flagellate other than that which develops from the parasites of Kala Azar or Oriental Sore. Bugs, both nymphs and adults, were repeatedly fed on cases of Kala Azar in whose peripheral blood parasites were either numerous or scanty. They were dissected at varying intervals after their last feed, and in a few of these bugs the parasite was recovered in an unchanged condition in a leucocyte, as well as free forms shewing some changes towards flagellation. These experiments were carried out at a temperature of from 80° to 82°F. In my second report I described some further experiments with *Cimex rotundatus*. Twelve adult bugs were fed on a case in whose peripheral blood 359 parasites were found in one film, and on dissecting them at varying intervals up to the fifth day, all the forms, from the unchanged parasite up to the fully developed flagellates, were recovered from the midguts of the bugs. The following is a summary of these changes:—“On the second day after being ingested by the bug the parasites begin to develop, their protoplasm increasing in volume; at the same time the macro-nuclei enlarge and shew signs of commencing division. The parasites may now either pass on to flagellation, or further growth, followed by the consecutive division of the macro- and micro-nuclei, may result in the formation of rosettes. The single flagellates enlarge and begin to divide longitudinally to result in oval or spindle-shaped cells; while the rosettes after flagellation begin to divide up into separate elongated flagellates. The oval or spindle shaped parasites divide repeatedly either by equal or unequal longitudinal division and result in smaller and more irregular forms. All these changes may be seen in the bug during the first three days. Still later, longitudinal division of the oval and irregular flagellates progresses rapidly, so that by the fifth day the majority have become small or spirilla-like flagellates.” In connection with these experiments it is necessary to draw attention to the fact that in the first series, the bugs were repeatedly fed, whereas in the last they were only fed once, and the last bug was

dissected on the fifth day. My reason for drawing attention to the number of times the bugs were fed will be seen later.

Donovan has attempted to confirm my observations on the development of the parasite in *Cimex rotundatus*. On three occasions he fed bugs on suitable cases, but states that he found no developmental changes in the parasites ingested, even after a week or ten days. He gives no precise details of these experiments. Being dissatisfied with his attempts to infect *Cimex rotundatus* he has searched for some other blood-sucking insect of local distribution, and one which is not so ubiquitous as the bed bug. He has succeeded in finding *Conorrhinus rubrofasciatus* de Geer, which he considers to be the most likely transmitter of the disease. He notes that both male and female *conorrhini* can be found which contain either digested or fresh mammalian blood in their midguts, and that both the adults and nymphs will readily suck up human blood. He records a flagellate of the genus *Crithidia* (*C. conorrhini*, Donovan) from the alimentary tract of the adult insect. He has fed this insect on suitable cases of Kala Azar, but has been unable to find any of the developmental forms of the parasite, and is now inclined to abandon the idea that it is the natural carrier. In connection with this insect I may point out that I have fed on myself the adult insect and several of the nymphal instars, and have found that the bite is most irritating. The actual puncture made by its proboscis is felt as a sharp sting, and about 12 hours later a large erythematous patch about the size of a shilling appears at the site of the bite; it remains in an inflamed and irritable condition for about a fortnight and then fades away, and a small nodule the size of a No. 6 shot persists for about one month. I therefore feel certain that were this bug a common blood-sucker in the Indian houses in George Town, it could hardly escape detection; further, the marked symptoms resulting from its bite could not possibly escape observation. In 1906 when I visited the houses of people suffering from Kala Azar, on enquiring what insects were in the habit of biting them, I never even heard of this *Conorrhinus*; it is a large coloured insect, and if it were a regular blood-sucker it would surely be well known. Recently I have endeavoured through several medical friends to obtain some information regarding its distribution in George Town, and as a result I have obtained a single specimen. On further enquiry I found that it never bites people, but is attracted by a light and will thus enter a house. I have however now had the opportunity of carrying out some feeding experiments with this bug, and these observations are described further on. This concludes the summary of all the experimental work which has been carried out in India with blood-sucking insects, and I will now give a critical review of the recent work on the closely allied form of Kala Azar of the Mediterranean littoral.

Feeding Experiments with the Parasite of Infantile Kala Azar.

In 1908 Nicolle and Comte made the important discovery that dogs in Tunis are naturally infected with a parasite indistinguishable from that of human Kala Azar. As the result of this discovery, Nicolle has advanced the hypothesis, that the Kala Azar occurring along the Mediterranean littoral is of canine origin, and that some ectoparasite of the dog is the invertebrate host. This hypothesis has been further elaborated by Basile who believes that the dog flea *Ctenocephalus canis* and probably *Pulex irritans* transmit the parasite from dog to dog, from man to man, from dog to man and from man to dog. Basile supports his statements by the following series of experiments:—

A number of dogs which were born in Rome, and whose bone marrow was examined for parasites, were taken to Bordonaro, and kept in a house in which cases of Kala Azar had occurred. Several months later the dogs living in Bordonaro developed Kala Azar, while the control animals in Rome remained healthy. Fleas (*C. canis*) taken from the infected dogs shewed flagellates in their intestinal tracts. Basile concludes that the dog flea is the invertebrate host of the parasite of canine Kala Azar. He next carried out a series of experiments to prove that this flea is the actual transmitter of the disease. Several fleas were taken from a clean dog, and placed in glass vessels, and kept at a temperature of 22°C. Later, a few of these fleas were placed in a vessel containing splenic juice from an infected dog, while the remaining fleas were kept as controls. The fleas which had been in contact with the infected material were later dissected, and an emulsion was made of the midgut contents of some of the fleas, and it was then injected subcutaneously into a young dog, which Basile states was clean. Smears were made from the gut contents of the remaining fleas which had fed on the splenic juice, and on being stained they were found to contain numerous developmental forms of the parasite; the control fleas were all negative. Fifteen days later the young dog which had been inoculated with the emulsion from the guts of the fleas developed signs of infection. On the 29th day the dog died, and it was found to be heavily infected; Basile concludes that the dog flea, *C. canis*, is the invertebrate host of the parasite of canine Kala Azar, and that in the same way *P. irritans* will probably be found to be the transmitter of Infantile Kala Azar. In 1911, continuing his experiments, Basile believes he has succeeded in infecting two young dogs, and an older one, by keeping them in a kennel with a dog suffering from Kala Azar; fleas which were abundant on the infected dog passed over to the experimental ones. Thirty days after the experiment was begun, hepatic puncture was performed on the three dogs, and they were found to be infected; two dogs kept as controls remained healthy. Basile concludes

that this experiment strongly suggests that the fleas from the infected dog carried the infection to the three, presumably, clean ones. In his next experiment Basile records having infected four dogs in Rome, by placing on them fleas, *C. canis*, and *P. irritans*, obtained from Bordonaro. He claims that the dogs used in this experiment were clean, and that there was no opportunity of their becoming infected before the experiment was begun. The fleas were placed on the dogs on December 1st, and early in March they all died of Kala Azar; two control dogs remained healthy. He concludes that this rigorous experiment proves that canine Kala Azar is transmitted by *C. canis* and probably by *P. irritans*. As the result of the examination of one thousand fleas taken off dogs, and from the beds of people in Bordonaro, he found four fleas to be infected with the parasite, and he believes it passes through its extra-corporeal life history in *C. canis*. The stained preparations of the intestines of these fleas shewed the pre-flagellate and post-flagellate stages and that these were identical with the figures given by Rogers of the cultural forms of the parasite of Indian Kala Azar. It should here be noted that Basile does not state whether he found the unchanged parasite in a leucocyte, the degeneration of the leucocyte resulting in the liberation of the parasite, and the early development of the flagellum; nor does Basile make any precise statements regarding the part of the intestine of the flea in which the several stages of the parasite were found. It is important to know for instance where the post-flagellate stages were encountered, for their exact position in the alimentary tract of the flea would enable one, who has studied the natural herpetomonads of fleas, to distinguish them from the pathogenic forms which the flea may ingest.

Another transmission experiment is recorded by the same observer in collaboration with Visentini. A young dog born in the laboratory was isolated for about six months, when fleas *C. canis* and *P. irritans* from Bordonaro were placed in large numbers on it. About a month later liver puncture revealed parasites in small numbers. In still another experiment, Basile records the examination of fleas *P. irritans* taken from the bed of a child suffering from Kala Azar, and in the intestine of one, parasites identical with those previously seen in *C. canis* were found; here again no mention is made of the unchanged parasite in a leucocyte. Basile, La Cava, and Visentini, examined fleas taken from an infected dog living in a house in which there was a case of human Kala Azar, and found in one of them numerous parasites. Later, fleas were collected from both dogs and human beings, and were placed on two dogs which were born in the laboratory, another dog of the same litter being kept as a control. About three months later the dogs began to shew signs of having contracted the disease, later still both died and at the autopsies varying numbers of parasites were found in their organs; the control dog was again found to be healthy.

Basile next proceeded to study the life history of the parasite in *C. canis* and *P. irritans*. He endeavoured to infect them by placing them in contact with cultures, of both the canine and human parasites, but the only result was the finding of degenerating flagellates in their midguts. Several other experiments were carried out by placing the cultural forms on various media, but the results were totally negative; that is to say, that though the fleas had, presumably, ingested the flagellates, these were unable to maintain themselves in the fleas and soon died. Basile states that this method of infecting fleas is unsatisfactory. He further maintains, that these negative results, as well as those of Franchini, are not conclusive, but that he has no doubt that the dog flea *C. canis* is capable of transmitting the parasite. It is difficult to understand why, if Basile is sure that the dog flea is the invertebrate host, fleas were not fed in the natural way on heavily infected dogs, and then dissected, and a search made for the unchanged parasite, and its development up to the flagellate stage.

Sangiorgi is the next observer who has examined blood-sucking insects for the developmental forms of the parasite. A large number of bugs, presumably *Cimex lectularius*, taken from the houses of people suffering from Kala Azar, gave negative results. In fleas, *C. canis*, taken from dogs caught in the town of Catania, he was able to recover in a single flea a large number of flagellates resembling the cultural forms of the parasite of canine Kala Azar. He concludes that these are the developmental forms of the canine parasite. Later, out of 378 fleas collected from dogs, fourteen were found to be infected with flagellates. In another paper Sangiorgi records the infection of a dog in Turin, which was contracted by coming in contact with an infected dog from Tunis, and in two out of thirty-nine fleas (*C. canis*), taken from this dog shortly before its death, flagellates resembling those previously described by this author, were found. Sangiorgi considers this transmission experiment to be conclusive. Basile in his most recent note states that he has found in *P. irritans* in Catania, a flagellate identical to that described by Sangiorgi from *C. canis*. Franchini has carried out some experiments with *Cimex lectularius*, *Pulex irritans*, *Ctenocephalus canis*, and *Pediculus capitis*. All these insects were made to feed on cultures of the parasite (presumably of human Kala Azar), but he states he has failed to find any of the parasites or their developmental forms in their midguts. He next turned his attention to mosquitoes, and utilised a species of *Anopheles* of the *claviger* variety. These insects were placed in glass jars, at the bottom of which there was a ten to fifteen day old culture, which was covered with thick gauze. On dissecting these mosquitoes, numerous flagellates were found after an interval of half to two hours, and from five to eight hours they were rare. After ten to twelve hours they were seen to have lost their flagella, and after twenty-four,

thirty-six, and forty hours, small round and oval forms were seen, which Franchini states are identical with the parasite as it occurs in man. These experiments were conducted at a temperature of from 28° to 30°C. Franchini concludes that the parasite of Kala Azar is able to live and multiply in the alimentary tract of this species of anopheles, and that the living flagellates may be passed out in its excreta. He also states that the parasite persisted in the mosquitoes although they were heavily infected with bacteria, whereas *in vitro* these organisms are fatal to its development.

The last observations it is necessary to quote are those of Gabbi, Franchini and Scordo. In some recent experiments, they state they have failed to infect both dog and human fleas with the parasite of human Kala Azar, and that the latter degenerate when placed in culture with the gut contents of these fleas; the bacteria, which may be present in the intestinal tracts of fleas, invariably destroy the parasites. These experiments at once recall those of Basile but the results are totally divergent. Gabbi has also carried out some further experiments by placing starved fleas in contact with spleen juice from a human case of Kala Azar, but he has not been able to observe any developmental changes. Again the examination of fleas taken from the beds of children suffering from Kala Azar, have failed to shew any flagellates. Gabbi, as a result of these experiments, doubts whether infantile Kala Azar is of canine origin, and concludes that neither the dog flea or the human flea, play any part in the transmission of canine and infantile Kala Azar.

I have followed these experiments with the greatest interest, and as I have for some time studied the natural flagellates of fleas, I am in a position to make some critical remarks regarding the experiments noted above. To any one who is not intimately acquainted with the many pitfalls which exist in connection with this work, the transmission experiments which Basile records would lead them to conclude, that canine Kala Azar and the infantile form are one and the same disease, and that the dog flea, *C. canis*, and possibly the human flea, *P. irritans*, are the true invertebrate hosts of the parasite.

I will now endeavour to show, that in spite of Basile's apparently conclusive transmission experiments with fleas, there is yet very little convincing proof that infantile Kala Azar is of canine origin, and that the dog flea is the true invertebrate host; in short, the problem of the etiology of both canine and infantile Kala Azar has yet to be solved. I will first deal with the flagellates which have been found in the dog and human flea in Italy. It is now universally recognised that fleas in many parts of the world are infected with natural flagellates, which have no connection with any blood parasite. A better example could not possibly be given than *Crithidia pulicis*, Porter, whose life history has been accurately described by Miss Porter; her experiments were

carried out with such precision that there can be no possible doubt regarding the nature of this flagellate. Unfortunately the complete life history of a flea herpetomonas is not yet known, though Swingle has described much of the life history of *Herpetomonas pattoni*, Swingle. I have had the opportunity of studying the flagellates of two species of fleas, namely, in *Ctenocephalus felis*, the dog, and the cat flea of Madras, and in *Ceratophyllus alladinis** the flea found on the squirrel, *Funambulu palmarum*. In *Ctenocephalus felis*, I have found that there are two distinct types of herpetomonads, one which is exclusively found in the malpighian tubes of the larva and adult, insect, and the other in the midgut of the larva and mature flea. The species which occurs in the malpighian tubes of the larva, though it possesses a flagellum is only slightly motile, while the form which is found in the midgut is highly motile, and is almost indistinguishable from the flagellated forms of the parasites of Kala Azar. In *Ceratophyllus alladinis*, on the other hand, I have only found the non-motile form which occurs in the malpighian tubes of the larva and adult insect. Although thousands of fleas have been examined I have unfortunately not yet been able to study their complete life histories, enough however is known to shew that extreme precaution is necessary before drawing any conclusions regarding the origin of these herpetomonads. It is important to note, that in the case of both these fleas the infection is acquired by the larvæ, and this emphasizes the futility of conducting experiments with fleas without first studying their natural flagellates. If this precaution is overlooked, the observer who finds any of these natural flagellates in fleas fed on mammalian blood, is very apt to conclude that they represent the extra-corporeal life cycle of a flagellate ingested by the fleas. Now, throughout all the flea experiments noted above, not a single observer records the examination of larval fleas, and I believe that, had this precaution been taken, there would to-day have been no doubt as to the nature of the flagellates found in *Ctenocephalus canis* and *Pulex irritans*. The fact that these parasites were not found in control fleas is of no value, for I have examined over five hundred larvæ of *C. felis*, belonging to a particular batch hatched out from eggs laid by one lot of fleas, with negative results, while in another batch of the same larvæ quite a number were found to be infected; the number of infected larvæ must depend on the number of infected fleas. The larvæ of both the fleas I have studied, invariably contain digested blood which is passed out by the adult insects in their excreta, and this at once explains how the larvæ become infected with these herpetomonads.

I have therefore no doubt that Basile, Sangiorgi, Alvares, and Pereira de Silva, have been dealing with one or more species of natural herpetomonas of fleas, and that these parasites have been mistaken for the developmental forms

* I wish to take this opportunity of thanking the Hon. N. Charles Rothschild for identifying this flea.

of the parasites of Kala Azar. That this is the case, is clearly shown by Marzocchi's observations in Piedmont where canine Kala Azar is unknown, for he states that he has found a flagellate in fleas taken from dogs not infected with Kala Azar. It is obvious then that the dog flea in Italy contains a natural flagellate, and it is most probable that *P. irritans* will be found to be infected with *Crithidia pulicis*. From what I have stated above it should be borne in mind that any flea may contain two distinct flagellates, and it is to be hoped that those who have the opportunity of studying these forms will not be satisfied by merely recording parts of their life histories, but will describe them in detail. In the past this has only been done in a few instances, and as a result, the life histories of most of these insect flagellates have been confused ; only certain stages, those most easily obtained, have been recorded, and new generic names such as *Leptomonas* and *Leptotrypanosoma* have been unnecessarily given them. I will have more to say on this subject on another occasion. Lastly, those who are endeavouring to solve these complicated problems in Tropical Medicine, have been led astray by such unwarranted statements as have recently found their way into the International Catalogue of Scientific Literature ; I refer to Woodcock's remarks there and elsewhere, that flagellates found in blood-sucking insects belong to the extra-corporeal life histories of vertebrate trypanosomes. The flagellates of blood-sucking insects constitute one of the most serious pitfalls in connection with this work, and I cannot too strongly urge upon those who may encounter them in their feeding experiments, to exercise the utmost precaution in accepting Woodcock's statements. I have no doubt whatever that those flagellates parasitic in the vast majority of blood-sucking insects have no connection with any blood parasite.

It is next necessary to shortly refer to Franchini's recent experiments with anopheles mosquitoes. This observer believes he has solved the problem of the method of transmission of infantile Kala Azar : it is however somewhat curious that no mention is made of the writer's feeding experiments with *Neocellia stephensi*. Franchini's experiments were conducted under extremely artificial conditions, for his mosquitoes were fed on blood containing the cultural forms of the parasite, and they are in no way comparable with those of the writer carried out in Madras. In Franchini's experiments there is no certain proof that the parasite of infantile Kala Azar will pass from its pre-flagellate to its flagellate stage in the mosquitoes, for the parasites were already flagellates. If it can be shewn, that in this particular anopheles in Italy the parasite will develop from its pre-flagellate to its flagellate stage, it will then be possible to consider Franchini's hypothesis as proved, but until this decisive proof is forthcoming it must surely be left *sub judice*. In my experiments with *Neocellia stephensi* in Madras, I was unable to observe any changes towards the development of the

parasite ; and moreover the method of spread of Kala Azar in Madras is strongly opposed to the view that the parasite is carried by a flying insect.

Further, there is no proof that Franchini has actually observed the post-flagellate stage of the parasite, for he makes no mention of the possibility of his mosquitoes being infected with a herpetomonas. In Madras, *Neocellia stephensi* is infected with a species of herpetomonas which is transmitted through the larva, and it is very similar to the parasite of Kala Azar. Franchini's mosquitoes were apparently caught at large, and not bred from the egg, which is the only possible way of excluding a natural herpetomonas. An important point to note in connection with this flagellate of anopheles mosquitoes is that it may pass back to its post-flagellate stage in the midgut of the insect. Recently when in Bombay, Dr. Bentley kindly shewed me in the fresh condition, the midgut of an anopheles which was packed with the post-flagellate forms of this herpetomonas and I have long known that these mosquitoes may pass out the flagellate stages in their excreta. From my observations on the life history of the parasite of Indian Kala Azar in *Cimex rotundatus* and *Cimex lectularius*, I find it difficult to understand how the allied parasite undergoes the final stages of its development so rapidly in anopheles, and how it can again pass back to man through the excreta of mosquitoes.

I will now shortly mention some points in connection with Basile's transmission experiments with fleas. In the first place it is necessary to note that canine Kala Azar is widespread in Italy, and as it is a comparatively benign disease, it is difficult to exclude it in such experiments. Secondly, I note that Basile makes no mention of the possible confusion between the parasite of canine Kala Azar and *Piroplasma canis* in the case of his dogs. I do not in any way wish to throw doubt on the accuracy of his observations, but they would have been much more convincing had this common piroplasm of dogs been mentioned. It is however by no means a remote possibility, for I have repeatedly seen *P. canis* in the spleens of dogs, morphologically indistinguishable from the parasite of Kala Azar. When inoculating dogs with the Indian parasite, this possibility was foreseen and every precaution was taken to exclude it, not only by examining the blood of the dogs for a considerable time, but by removing all ticks. In this way I failed to obtain any positive results, though one of the dogs was kept for many months.

The Life History of the Parasite in *Cimex rotundatus* and *Cimex lectularius*.

For several months before the experiments I am about to record were begun, adult bugs both *Cimex rotundatus* and *Cimex lectularius* were collected,

and fed in the laboratory. Their eggs were separated, and the first nymphal instars of each species were obtained. The adults of *Cimex rotundatus* were collected in Saidapet, while those of *Cimex lectularius* were imported from London; I wish to take this opportunity of thanking Miss Blakeney of the Mildmay Mission Hospital for the great trouble she took in obtaining this species for me. As before, the bugs were kept on pieces of sterilised filter paper in glass tubes, and were thus readily fed on any part of the body.

On January 26th I was informed that there was a suitable case in the General Hospital, and on visiting the patient I examined a film of finger blood and found in it 23 parasites. The following is a short history of the case:—Mr. R., aged 40, a Eurasian of independent means, had been suffering for the last six months with low fever, and he noticed that he was slowly getting thinner. About the end of December 1911, he developed an attack of diarrhœa, with great pain in the region of his large intestine; he gradually became worse and the diarrhœa now was more of a dysenteric nature, blood and mucus being frequently passed with much tenesmus. As he began to get rapidly worse, and was having about thirty motions in the twenty-four hours, he sought admission in the General Hospital. On examining him I found that his spleen was slightly enlarged, while the lower margin of his liver could just be felt on deep inspiration below the costal margin. On palpating his abdomen he complained of great pain all over the region of his large intestine. It was noticed that he was a man of good physique and, although there was evidence of considerable wasting particularly in his arms and legs, his body was well covered; his general condition was favourable, and there was every chance of his recovering from this acute attack of dysentery. His motions were foul smelling and chiefly consisted of green sloughs with blood and mucus. His breath had that characteristic fœtid odour, so often noticed at this stage of the disease. On January 27th another blood film was examined and thirty-one parasites were found. On this day a large number of the first nymphal instar of *Cimex rotundatus*, contained in two tubes, A and B, were fed on the patient's arm. He was not seen again till the 29th, when a blood film was taken and 100 parasites were found. On the 30th the two tubes of nymphs, A and B, were again applied and, most of them fed for the second time; a fresh batch of nymphs of *Cimex lectularius*, tube C, were fed for the first time as well as a large number of nymphs of *Conorrhinus rubrofasciatus* (tube D). On the 30th it was noticed that the patient was decidedly worse, he was much weaker, and was passing his motions in bed. On the 31st a blood film was taken and 250 parasites were found, and on this day the bugs in tubes A, B, C and D were again fed, as well as those in tube E, a fresh batch of *Cimex lectularius*. Late in the same evening the patient became rapidly worse and died the next day.

It is important to note exactly when the several batches of bugs were fed. Two tubes A and B, containing nymphs of *Cimex rotundatus*, were first fed on R. on January 27th, again on the 30th and 31st. One tube C, containing *Cimex lectularius*, was applied on the 30th, and again on the 31st. The nymphs of *Conorrhinus rubrofasciatus*, contained in tube D, were first fed on the 30th, and again on the 31st, while the nymphs *Cimex lectularius* in tube E were fed only once on the 31st. It should again be noted that all these bugs were bred in the laboratory, and that they had their first feed on the patient. It will be seen then that these experiments were carried out with the utmost precision, and under natural conditions.

In order to explain more clearly the results which have followed these feeding experiments, it is necessary to again refer to my previous work on this subject. It will be remembered that in 1907 I repeatedly fed bugs on one or more cases for several days, and then dissected them after varying intervals. In experiment 1, noted on page 13 of my first report, bugs were fed on case 11 on March 29th, again on the 30th, 31st and April 2nd. On the latter day one bug was dissected and its midgut was examined in the fresh condition, and a flagellate was found, but I then noted that its movements were slow and in no way similar to that of the natural herpetomonads of mosquitoes. I was then not aware of the significance of these slow movements of the flagellate stage of the parasite in the bug, as compared with those of the mosquito herpetomonads.

In experiment 1, case 12 noted on page 14 in the same report, twenty-two bugs were fed on the case on May 16th; they were again fed on the 17th and 18th, and on the 19th one bug was dissected, and in its midgut contents, two parasites were found which had probably been ingested on the 18th. The remaining bugs were next fed daily from the 19th to the 31st, and on June 1st one bug was dissected, which had last fed on the 31st, and fifteen parasites were found in the film; some of these shewed the earliest changes towards development. On June 4th another female bug was dissected, and a single parasite was found which had still further developed. On June 4th the remaining bugs were again fed on another case of Kala Azar, and on dissecting four bugs on the same day, no parasites were found in them. On June 5th another bug was dissected and eleven parasites were found, but unfortunately no note was then made as to whether this bug had fed on the previous day. The remaining bugs were dissected on the 5th and 6th with negative results. In the last experiment recorded in my first report, six bugs were fed three times on a case and then five times on a monkey; on dissecting these bugs three unchanged parasites were found, but no developmental forms. In the experiment recorded in my second report, twelve adult bugs were fed on the case, and the last bug was dissected on the fifth day, none of the bugs having been fed again in the interval.

At this stage of my experimental work, I was under the impression that the parasite, after flagellating in its invertebrate host, passed back to man in the form of the spirilla-like parasites described by Leishman ; I believed this would either take place by regurgitation or by the entrance of these forms into the salivary glands of the bug. Since then I have learnt that this view was entirely erroneous, and that the spirilla-like forms are not the final stage in the extra-corporeal life history of the parasite. By studying the life histories of many insect herpetomonads, I came to the conclusion that, in order for this human parasite to find its way back to man, it must be injected into his body in a non-flagellated form, the post-flagellate stage. In my earlier experiments I aimed at obtaining a massive infection by feeding my bugs repeatedly, so that I could the more readily find the infective stages either in the midgut of the bug, or in its salivary glands ; but, as will be seen later, by the very methods employed, I failed to prove anything more than that the parasite flagellates in the bug. Later it was thought that one feed was sufficient, and that if the bugs were fed again the infective stage might be injected into the next person the bug fed on. Here again I failed to prove anything more, and only found that the development in the bug may go on up to the fifth day without shewing the infective forms to be the predominating ones.

Though convinced that the bug was the true invertebrate host, I was dissatisfied with the results obtained, and felt that there was some fact in connection with the development of the parasite in the bug which was yet to be discovered. On turning my attention to the method of the spread of Kala Azar in Madras, I could not help thinking there was some natural obstacle which frequently prevented the parasite from completing its life history in the bug ; this was the only satisfactory explanation of why the disease does not tend to spread outside the endemic area. Precisely the same was noted in the case of the parasite of Oriental Sore in Cambay, and, as a result of my work on this parasite, I came to the conclusion that the natural obstacle was connected with the digestion of the blood in the bug ; for instance it was soon found out that temperature had a marked effect on the digestive processes in this insect. It was then thought that the problem might be solved by studying the effect of blood on some natural herpetomonads of a blood-sucking insect. Such a parasite was found in *Herpetomonas culicis*, parasitic in *Culex fatigans*, and it was noticed that the parasite could be readily found in the male mosquito, but rarely in the female. On investigating this question further, it was found that when female mosquitoes were bred out from larvæ, the majority of them were infected, and when they were fed on human blood, the parasites almost invariably disappeared, in a few however they were recovered. Although a satisfactory explanation of this disappearance of the flagellates was not at once

forthcoming, it appeared as if the blood had some injurious effect on the flagellate stage of the parasite. I had just finished these experiments with the mosquito herpetomonas, when I heard of the suitable case of Kala Azar in the Hospital and bugs were fed as noted above.

On February 1st, 1912, one of the nymphs of *Cimex rotundatus*, from tube A, was dissected; this bug had first fed on R. on January 27th, and for the last time on the 30th. In the smear, made from its midgut contents, the early stages of the development of the parasite were found consisting chiefly of free parasites and several shewing the commencing formation of the flagellum. On the same day a nymph of *Cimex lectularius* from tube E was also dissected, this bug had fed only once on R. In the film, much the same parasites were seen, except that the multiplication appeared to have gone on more rapidly, for several young flagellates were found. On February 2nd another nymph of *Cimex rotundatus*, tube A, was examined, and now all the parasites were well advanced towards flagellation, and there were many mature flagellates. On February 3rd I decided to give the bugs in tubes A, B and C, another opportunity of feeding. A case of Kala Azar was selected, in whose peripheral blood there were only a few parasites to be found, six were seen in a blood film on this particular day. About half of the bugs in the several tubes fed well on this case. On February 4th one of the nymphs from tube B was examined, it had not fed on the second case on the 3rd. In the film a number of flagellates as well as younger stages were seen. On February 5th a specimen of *Cimex lectularius* from tube C was examined, and a large number of mature flagellates, as well as many shewing multiple segmentation were found. On the same day one of the nymphs, *C. rotundatus*, which had fed on the second case on the 3rd was examined, and except for two unchanged parasites none of the early developmental forms or flagellates were seen. A number of other specimens of both species, which had fed on the second case on the 3rd, were next examined and it was at once found that all the parasites had disappeared and only the ghosts of some flagellates could be recognised. Several bugs, which had not fed on the second case, were then examined and in them all, the further developmental forms were readily found, and in several of the bugs an enormous multiplication had taken place, and masses of rosettes were seen. It was at once recognised that the long suspected natural obstacle was discovered, and it was now quite clear that the younger developmental forms and the flagellates, which may be present in a bug, are destroyed when it takes a fresh feed of blood; in this case it was the blood from a patient suffering from Kala Azar. Two nymphs of *Cimex lectularius*, tube E, were next fed on a young monkey, *Macacus sinicus*, and they were examined exactly twelve hours later. Here again all the developmental forms, which had been unmistakably present

in these two bugs, were completely destroyed, and many groups of flagellates could just be recognised as such. There could now be no possible doubt that there is some body, present in man's blood, as well as in that of the monkey, which is inimical to the development of the parasite.

This discovery at once explains some of the most puzzling questions in connection with the disease and its parasite. Looking back at my previous attempts at infecting the bug, it will be seen that, by repeatedly feeding the insects, I was only destroying any developmental forms already present, and that in this way massive infection of a bug could never be produced; the failure to attain this object was at the time most disappointing. Had I only kept the bugs utilised in the experiment recorded in my second report five days longer, it is very probable that I would then have discovered the complete development of the parasite.

This new discovery explains another point in connection with the parasite in man, which so far as I am aware no one has yet attempted to solve. Why is it that the parasite of Kala Azar never becomes a flagellate in man's blood, or his organs? There must be many occasions when it is free in the blood, and it should then develop into a flagellate. Further, it is evident that the white blood cells are incapable of destroying the parasite, this being the case there is no reason why it should not enlarge and become a flagellate while still in the cell. This difficult question is I believe at once explained, by the discovery that human blood has some body in it which not only prevents the process of flagellation, but also destroys the flagellates. This substance is in all probability the complement, and it is well known that it is itself destroyed in about two days, when blood is drawn from the human body. This fact further explains why the parasite only begins its development in the bug on the second day. It may also explain why Kala Azar of the Mediterranean littoral only attacks young children and is rarely met with in adults. Lastly this discovery offers, in my opinion, the only satisfactory explanation of the curious fact that Kala Azar in Madras hardly, if ever, spreads outside its endemic area. Assuming that Kala Azar is transmitted from man to man by some blood-sucking insect, why should the disease cling to one spot in a town and not spread, as do other insect-borne diseases? It is only right that an attempt should be made to answer this question. There must be innumerable opportunities for the true invertebrate host to become infected in large numbers, and there is no apparent reason why they should not carry the parasite outside Madras. This puzzling fact in connection with the disease can now be better explained, when it is known that though many bugs may become infected, only those which do not feed again till the parasite has passed back to its post-flagellate stage are infective; the number capable of transmitting the parasite must then necessarily be reduced.

Exactly the same will in all probability hold good for the parasite of Oriental Sore, for as I observed the disease in Cambay, I was struck by the fact that it has never spread outside the town itself.

I will now describe the life history of the parasite as I have studied it in *Cimex rotundatus* and *Cimex lectularius* fed only once on the patient.

As already stated, the bugs were kept in glass tubes, and throughout the experiment were placed in a cold incubator at 22° to 24°C. They were dissected in the way I have already described, and the films when smeared out, were fixed with absolute alcohol and stained with Romanowsky's stain.

The Pre-flagellate Stage in the Bug.

The parasite is ingested by the bug, either in a white blood cell, or in a macrophage, and it remains in an unchanged condition from about 36 to 48 hours. Figures 1, 2, 3, and 4, on the plate, depict several parasites in white blood cells from the bug; the leucocytes in each case shew commencing disintegration as evidenced by the vacuoles seen in their protoplasm. While still lying in a leucocyte, the parasite may shew the earliest developmental changes, it enlarges, its protoplasm becomes vacuolated, granular and more voluminous and now takes on a marked blue colour when stained with Romanowsky's stain. At the same time the nucleus enlarges and stains a lighter pink, and may contain a single central chromatic granule, or a number scattered about. The nucleus at this stage may lie at one end of the parasite, or about its centre. The blepharoplast is now distinctly enlarged, and may lie close up to the nucleus or more towards the periphery of the cell. The parasite at this stage usually retains its original shape, and measures from 4 μ to 6 or 7 μ in length, and from 3 μ to 4 or 5 μ in breadth. Figure 5 is a young growing parasite still lying in a leucocyte, while figure 6 represents a free form and figure 7 another large one lying at the margin of a disintegrating white cell. When looking over a film made from the midgut contents of a bug, these early developing forms can at once be recognised, they are extremely characteristic, their size, together with their granular blue staining protoplasm, render them very striking objects, and they cannot possibly be mistaken for anything else. Before enlarging further, many of these young forms exhibit the earliest signs of binary fission, their nuclei become kidney shaped and the chromatic granules may be seen grouped together in two halves. Figures 8, 9 and 10 shew this appearance, while in Figure 11 although the nucleus is still more or less round, the blepharoplast is elongated and thickened. As further growth takes place, the nucleus may divide, as seen in Figure 12, or the blepharoplast may first enlarge and then divide, Figure 13. At this stage the parasite itself may exhibit the early

appearances preceding simple binary fission ; its body becomes indented, usually at the end furthest away from the nucleus, and it later splits into two.

The Flagellate Stage.

It will be remembered that, in my second report, I pointed out that at this stage of the life history of the parasite, one or other of two changes may take place ; (1) the parasites may rapidly pass on to flagellation ; or (2) what is more important, they further enlarge and by consecutive division of their nuclei and blepharoplasts produce a rosette of from four to eight parasites. I will first follow the changes undergone by the single forms as they approach flagellation. About the time the nuclei are going to divide, there appears a small pink staining area between the blepharoplast and the margin of the cell ; it is from this structure that the flagellum is developed. In my second report, I considered it was of the nature of a vacuole, and that the flagellum grows out of it as evidenced by the pink staining strands seen lying across it. Since then I have made a special study of the formation of the flagellum, not only in this parasite, but in the allied parasite of Oriental Sore and in many natural herpetomonads, and am able to give a more satisfactory explanation of its nature. From a careful study of the formation of the flagellum at its earliest stage, it was seen to grow out from an already existing pink staining filament. Any one who has studied deeply stained specimens of the parasite of Kala Azar will have noticed that many of them have a pink filament close to, but not touching the blepharoplast, and which stretches towards the periphery of the cell. This pink filament may be straight or slightly bent, and usually lies perpendicular to the blepharoplast. Christophers was the first observer to draw attention to this filament and he has clearly depicted it. Since then several observers have described it from the parasite of infantile Kala Azar and that of Oriental Sore. Novy speaks of it as the rhizoplast.

In many of the bugs, both *Cimex rotundatus* and *Cimex lectularius*, I have had the opportunity of examining a large number of parasites exhibiting the earliest growth of the flagellum. Figure 18 depicts a parasite in which the pink filament is merely thickened, while figures 12, 13, 14, 15 and 19 shew the various appearances just prior to the extrusion of the flagellum. As the pink band thickens more and more, it appears as a round or oval area, and extending across it there may be one or more thin filaments. This vacuole-like appearance is I believe nothing more than the enlarged filament stretched out as the parasite adheres to the slide. Later, the flagellum, which appears to consist of several delicate filaments, protrudes from the body of the parasite and then becomes free. The question now arises, what is the nature of the pink staining

filament as seen in the pre-flagellate stage of the parasite? There can be no doubt that it is directly connected with the development of the flagellum, but the view that it represents the root of this organ does not explain its true nature. It is at present generally believed that the rhizoplast or root of the flagellum is in some way connected to the blepharoplast or so-called kineto nucleus, and that the flagellum arises *de novo* from the blepharoplast. As a result of my studies I believe, on the contrary, that the flagellum is of ectoplasmic origin, and that it grows from the pink band or filament present in the pre-flagellate stage and that this structure is a myoneme. It is well known that many trypanosomes have myonemes, or contractile bands, and I have seen them well displayed in *Herpetomonas muscae domesticæ*; in most other herpetomonads, however, they are so delicate that they cannot as a rule be seen. In many of the forms I have studied, the pink filament may not only be seen in one part of the parasite but can be found stretching right across it. In smears made from the spleen of a case of Kala Azar, or from the blood of an Oriental Sore, I have frequently seen parasites which have ruptured, the protoplasm and nucleus having been extruded, and the ectoplasm then stains pink, and a well developed band can be seen stretching across the cell; very often the blepharoplast remains, and is then seen lying at right angles to the pink filament. Wenyon depicts this appearance (ref. fig. 28, plate 12) but he considers it to be an abnormal form. In support of the view that they are merely ruptured parasites, from which the protoplasm and nuclei have been extruded, it is only necessary to state that lying beside many of them there were the nuclei and protoplasmic debris.

The view that the flagellum grows from a myoneme, and is therefore of ectoplasmic origin, can readily be demonstrated in these parasites by studying its early development, whereas it has never been seen to develop out of the blepharoplast; further I have been unable to find it directly connected with this rod-shaped structure. I hope on another occasion to return to this subject and to depict the several appearances in greater detail.

From a reference to figures 12, 13, 14 and 15, it will be seen that the nucleus may divide first, or on the other hand the blepharoplast, and that the flagellum may begin to be formed before the parasite itself has divided. These single enlarged parasites may measure from $7\ \mu$ to $8\ \mu$ in length, and from $5\ \mu$ to $6\ \mu$ in breadth. At this stage these forms may either divide and then flagellate, the two flagella developing side by side, or they may increase in size, and after the nuclei and blepharoplast have divided, the parasite may itself split into two. The flagella become extruded soon after the parasites have separated. When division of the parasites is about to take place, an indentation forms at one end, and the line of division passes down, and the parasites may only remain attached by their flagella, or by both poles. Whatever type of development

takes place, the same result is produced, namely, two oval flagellates lying side by side.

Some of these single parasites, instead of at once dividing, elongate and result in the formation of spindle-shaped cells; this appearance is seen in figures 20 and 26. The spindle-shaped flagellates themselves begin to divide, and they can then be seen in pairs in all stages of divisions as depicted in figures 16, 17, 24, 25, 26, 27, 28 and 30.

The second type of multiplication, which is very characteristic of the development in the bug, results in the formation of rosettes of from four to more flagellates. A parasite about to undergo this change, instead of at once dividing, enlarges, and becomes globular in shape, its nucleus and blepharoplast now divide, and these in turn divide again, and in this way there may be eight nuclei and eight blepharoplasts. It is not uncommon to find that either the nuclei have divided more rapidly than the blepharoplasts, or *vice versá*. At the same time the flagella may be seen in all stages of growth, or there may be only one flagellum. Figures 33 and 41 shew these changes and such cells may measure from 10 μ to 12 μ in length, and from 10 μ to 11 μ in breadth. As soon as the flagella have grown out, the parasites begin to separate and break away, figure 29.

All these developmental changes take place both in *Cimex rotundatus* and in *Cimex lectularius* from the third to the fifth day after the last feed of infected blood. The formation of the spindle-shaped or elongated flagellates may then take place in one of the following ways :—

- (1) A young growing parasite may flagellate, and without dividing, elongate and become spindle-shaped, but this is not the usual method of development.
- (2) A growing parasite, instead of at once flagellating, divides, and the resulting pair become elongated, and they may in turn divide again; or
- (3) A parasite instead of flagellating or dividing, enlarges, and the nucleus and blepharoplast divide up into eight or more, the flagella then grow out and the cell splits up into many elongated flagellates.

It should be noticed that many of the oval or spindle-shaped flagellates themselves divide up regularly or irregularly into several shorter or longer forms depending on the size of the parent cell. Figures 31, 32, 36, 37, 38, 39 and 40 depict these changes. The resulting flagellates, whether they be long or short, are very characteristic. Their protoplasm stains dark blue and appears to be granular and vacuolated; their nuclei, oval in shape, are usually situated about the centre of their bodies, they stain pink and contain many chromatic rods.

Their blepharoplasts may be rod-shaped or circular, and always lie closer to the nuclei than to the anterior ends of the parasites. Their flagella stain deep pink and consist of many delicate filaments adhering together, and arise from a point a little anterior to the blepharoplasts.

We have now seen that by the fifth day the parasites, have grown and multiplied, so that in place of the original pre-flagellate stage ingested in leucocytes, the parasites have not only become free, but they have increased in numbers and are now long actively moving flagellates.

The spindle-shaped or elongated flagellates, which predominate from the fifth day onwards, now begin to divide up and the rosettes are seen as masses with their flagella intertwining and directed inwards. In many of these spindle-shaped forms a process of unequal division was observed, such as was first described by Leishman from cultures *in vitro*. Parasites, which appear to be dividing normally into two more or less equal flagellates, exhibit this curious unequal division resulting in the splitting off of spirilla-like daughter cells. These may contain neither nuclei or blepharoplasts and frequently they only have one chromatin mass. I do not propose describing these parasites further, beyond remarking that several were seen both in *rotundatus* and *lectularius*; I will describe them more in detail on another occasion and will then explain what I consider to be their true nature.

In several of the bugs, particularly in *lectularius*, many large rosettes such as are depicted in figures 45 and 46 were seen, and the bugs contained a large number of flagellates, such as are shewn in figures 42 and 43. These mature flagellates measure from $12\ \mu$ to $20\ \mu$ in length and from $4\ \mu$ to $5\ \mu$ in breadth, their nuclei are nearly always oval in shape and lie about the centre of their bodies; the blepharoplasts may be rod-shaped or circular. The posterior ends of the parasites are pointed, while their anterior ends are rounded and their flagella measure about $24\ \mu$ in length. In my second report I stated that I was then unable to observe equal division taking place in these long flagellates, but I am inclined now to think that they may divide further in this way, but this division was not seen in many instances. In the rosette shewn in figure 46 there is some evidence that the parasites were dividing up in this manner.

By the sixth and seventh days the parasites were nearly all elongated flagellates, either free or massed together in rosettes. It should here be noted that several groups of flagellates were seen in the films, attached to cells by their flagella. Figure 40 represents such a group, and in a fresh preparation this was very clearly demonstrated; it is exactly similar to what takes place in the case of all insect herpetomonads as they approach their post-flagellate stage. Figure 44 requires special mention. Many of the spindle-shaped flagellates exhibited a curious appearance at their posterior ends as shewn in this figure;

a part of the protoplasm appeared to be breaking off, and in some it was seen as a mere tag. It was at first thought that this appearance was merely the result of the pointed end becoming twisted, but it was seen in many of these dividing forms, and I am at present inclined to think it is a portion of residual protoplasm being thrown off.

The Post-flagellate Stage.

The whole interest in the life history of this parasite, centres round the final stages in its development, the nature of which was predicted in 1907 ; it is true some observers claim to have seen this stage in cultures in the test tube, I however fail to understand how this is possible. Franchini records having found cystic forms in old cultures of the parasite of infantile Kala Azar, and in my opinion he rightly regards these as degenerating parasites. It is interesting to note that in several of the bugs which had a second feed on the other case of Kala Azar recorded above, such round degenerating forms were seen ; these had undoubtedly once been flagellates and the fresh blood had caused them to degenerate, and assume a rounded form. In the recent number of the Kala Azar bulletin on page 49, there is a note by the writer to the effect that Dr. Row has succeeded in tracing the final stages of the parasite of Oriental sore of Cambay in cultures *in vitro*. It remains to be seen how the morphology of the forms discovered by Dr. Row, correspond with those now to be described. It should be noted that, if in the bug fresh blood causes the flagellates to soon round up and degenerate, it is only natural to expect that exactly the same would take place when the culture containing flagellates is transferred to fresh medium, which presumably contains blood. The extreme sensitiveness of these flagellates of the parasite of Kala Azar to human or monkey's blood makes me doubt whether these rounded forms, found in old cultures of the parasites of Kala Azar, and Oriental sore, are anything more than degenerating forms.

The post-flagellate stage in the bug begins on about the eighth day, and appears to be completed by the twelfth. It was noticed that the mature flagellates on the seventh, eighth and ninth day altered in appearance, the blue staining protoplasm, which is characteristic of all the earlier forms, had now changed, and the flagellates were a light pink ; further many were now seen with short flagella as is depicted in figure 50. Several other important structural details left no doubt that the long looked for post-flagellate stage was at last found. Figures 47, 48 and 49 represent the earliest changes towards the final rounding up of the parasite. These flagellates are more or less oval in shape, and measure from 9 μ to 12 μ in length and from 5 μ to 6 μ in breadth ;

their protoplasm stains light pink, it is almost homogenous, and is surrounded by a well marked pink staining periplast. The other outlines of their nuclei are obscured and they are full of small pink rods ; their blepharoplasts stain as before. Most of these forms were undergoing division as evidenced by their having two nuclei, two blepharoplasts, and two flagella. Any one who has seen this stage and compared it with the younger growing forms depicted in figures 25, 26, 27, and 28, could not confuse the two. The young parasites stain blue and their nuclei are well defined. It may be thought that these rounding up forms are degenerating flagellates ; in order to disprove this view, bugs were examined at this stage in the fresh condition, and it was found that these forms were extremely active and that they were attached to the intestinal wall by their flagellar ends. When the gut was ruptured, they were seen lashing about while still attached. I have never before seen the flagellate stage of the parasite exhibiting such active movements, those I have previously seen only shewed slow movements ; this was obviously due to the fact that the bugs had fed again.

Like the post-flagellate forms of all other herpetomonads, the parasite of Kala Azar then begins this stage by attaching itself to the intestinal wall, and there it slowly rounds up and at the same time divides again. On the completion of this final division, smaller forms are produced, and these again divide, resulting in the final stage which is depicted in figures 67 to 73. Several of these rounding up parasites had long flagella, figure 61, while the majority only possessed short stumpy ones and many merely the remains of flagella projecting as faintly staining filaments ; figures 51 to 66 shew these appearances. Here again it may be said that such forms as are depicted in figures 52, 53, 54, 55, 60 and 62 are young forms about to flagellate. I have carefully described that stage of the parasite which proceeds the extrusion of the flagellum, and have drawn attention to its appearance. The only way the round post-flagellate resembles it, is in its shape, otherwise they are totally different. The smallest one I have seen is depicted in figure 67 and the majority are larger than the corresponding pre-flagellate stage. They have ill-defined nuclei which contain many granules ; the blepharoplast, which is also present, is not much altered. These forms stain well and have a distinct pink-staining periplast. In none of these parasites were any of the appearances seen which could possibly suggest that they were pre-flagellates just about to enlarge and flagellate. It is important to note that when they appeared in the bugs, they were the predominating forms, whereas just a few days previously, flagellates were the only parasites to be seen.

It is necessary now to shortly consider how the parasite finds its way back to man when the bug feeds again. The life history, I have described above,

takes place in the midgut of the bug, and throughout its development it never leaves it. I have so far searched in vain in the salivary glands of the bug for the post-flagellates; this stage appears to be completed in the midgut of the bug. How then can they be injected into man? This I believe takes place by regurgitation, as there is no structure in the upper part of the alimentary tract of the bug which could prevent this happening. The bug has no thickened proventriculus which could act as a constrictor and prevent any of the contents of the midgut from passing up the œsophagus into the pumping organ in its head. Further, the bug has the curious habit of returning to feed after it is replete with blood; and I have seen one insert its proboscis, no less than six times, before it was satisfied; all the stages of the bug behave in this way. On examining the first nymphal instar, after it has fully fed, under a low power lens it will be seen that the midgut is pressed right up into its prothorax, and that the bright red blood can be readily seen in its head. If such a bug contained the post-flagellates ready to be injected, they would tend to be loosened by the peristaltic movements which follow the ingestion of blood, and if the bug returned again to feed, there is no difficulty in understanding how they may be regurgitated into the wound. I do not intend however to try and explain further this part of the problem, for I hope in my next experiments to endeavour to solve it once and for all.

There is one point, which is specially brought out in these experiments, and that is, that the parasite will develop as readily in *Cimex lectularius* as in *Cimex rotundatus*; I have long wished to utilise this bug, but up to the present I have not succeeded in doing so. It was further noticed, that the development in the European species was, if anything, more marked than in its oriental ally, this may only be due to the fact that the digestion of the blood in it takes place more slowly, thus giving the parasites the maximum amount of nourishment. As a result of the experiments with *Cimex lectularius* I venture to predict that the closely related parasite of the Mediterranean littoral will also undergo its development in this bug. The experiments which have been carried out with this insect have, up to the present, been conducted under artificial conditions, and they in no way simulate those described above; the search for parasites in bugs caught at large is a waste of time, for the reason that though the bug may have fed on a suitable ease it may have fed too often with the result that all the flagellates may have been destroyed.

I find myself in entire agreement with Gabbi who believes that Infantile Kala Azar is not of canine origin, and consider that those who believe the contrary should bring forward much more convincing proof than has yet been produced.

In order that any weight may be attached to feeding experiments, both

with fleas and mosquitoes, it is very necessary for the observer to shew that he is aware of the many pitfalls which may be encountered in such work, and to take every precaution to exclude natural parasites of the insects. Further, I would emphasize this point, that before it is possible to accept a statement that the parasite of Kala Azar will multiply in any insect, it is of the first importance for the observer to shew that it actually develops from its pre-flagellate stage. This has up to the present not been done. Lastly I may say that I attribute all my success in this work to the valuable knowledge gained by studying insect herpetomonads; the working out of their life histories is almost of equal importance to the study of the extra-corporeal life history of the parasite of Kala Azar. I have studiously refrained from recording only parts of the developmental stages of these harmless flagellates, for owing to their great pleomorphism they exhibit such varying forms, that as a result, they have been much confused in the past and still less understood. They form, as a matter of fact, a much more homogenous group than do the *trypanosoma*. It is more particularly those forms which occur in blood-sucking insects which deserve the most careful attention of Protozoologists and students of tropical medicine. The fact that one, and possibly more than one, of these human herpetomonads is a true insect parasite in that it undergoes the most important part of its life history in its invertebrate host, should at once arrest our attention, for it is no imaginary hypothesis to think that any one of these, at present, harmless parasites, may become pathogenic to man or the domestic animals. The fact that the herpetomonas of anopheles mosquitoes may undergo its post-flagellate stage in the midgut of the insect suggests that at least this parasite is varying its life history.

The behaviour of the Parasite in *Conorrhinus rubrofaciastus*, de Geer.

Mesnil, commenting on my discovery of the development of the parasite in *Cimex rotundatus*, has expressed the view that it does not follow, that, because the parasite flagellates in this bug, it will prove to be the true invertebrate host; the development may merely take place, owing to the fact that the bug ingests a large quantity of blood, and that its midgut then simulates a culture tube. Wenyon has further elaborated this view, and in his report on Oriental Sore in Bagdad, when referring to the development of the parasite he has observed in the bug, he makes the following statement: "These observations are very similar to those made by Patton on the development of the parasite of Kala Azar in *Cimex rotundatus*. If the parasite of the sore can develop into a flagellate in the stomach of an insect, not its true host, then it would be expected that the very similar parasite of Kala Azar would develop in the same manner. Such experiments and results only shew that it is unsafe to draw the

conclusion that an insect shewing such developmental stages in its gut is the true carrier of disease, for there is no question of the possibility of the bed-bug being the carrier of the sore in Bagdad. This development is then merely a partial imitation of what would actually take place in the true intermediate host." In a still more recent note, Wenyon states that he "has been driven to the conclusion that the parasite of Oriental Sore develops in the intestine of the bed-bug in just the same way as in artificial cultures in various blood-containing media. In connection with Patton's discovery of the development of *Leishmania donovani* in the bed-bug Mesnil has made the same suggestion, so that such a development does not necessarily mean that the insect in question is the natural host of the parasite. It is highly probable that the parasites of the group *Leishmania* will develop to a certain extent in the intestines of many insects, provided that they find there a suitable pabulum rich in blood. The development I have described of *Leishmania tropica* in *Stegomyia fasciata*, and that of *Leishmania donovani* in *Anopheles* by Professor Franchini, may be of this nature, and only, so to speak, accidental, owing to the rich nutrient fluid taken up with the parasites by these blood-sucking flies." In connection with these statements it is first necessary to point out that the parasite of Indian Kala Azar will not flagellate in *Culex fatigans*, *Neocellia stephensi*, *Stegomyia suguens*, *Ornithodoros savignyi*, *Pediculus capitis*, and *Pediculus vestimenti*, in spite of the fact that these insects, particularly the mosquitoes and the tick, suck up large quantities of blood. Precisely the same was noted in the case of the parasite of Cambay Boil, it will not flagellate in *Stegomyia*. Further I very much doubt whether Wenyon actually observed the flagellate stage of the parasite of Bagdad Boil in *Stegomyia fasciata*. He admits having found a herpetomonas in the larva of this insect and it is inconceivable to think that if it is present in this stage it will not be present in the nymph or the adult insect; it is very probable that when the female mosquitoes fed on the boils the flagellates disappeared in much the same way as did those in *Culex fatigans* noted above, and that they only persisted in a few. The conclusive proof that the flagellates observed by Wenyon in *Stegomyia fasciata* were the developmental forms of the parasite they had ingested, is unfortunately wanting, for although he figures the early stages they are not sufficiently convincing; if the parasite regularly develops in this mosquito there would be no difficulty in following all the stages in its growth, from the unchanged parasite in a cell, up to the formation and extrusion of the flagellum, such as I have repeatedly observed in the bug. In the local *Stegomyia* of Cambay I failed to observe any development, and this mosquito never contained a natural herpetomonas, thus my failure to find flagellates or round forms.

There is therefore at present no proof that either the parasite of Kala Azar,

or that of Oriental Sore, will flagellate in any insect other than their true invertebrate host, and I am able further to shew that the parasite of Kala Azar will not flagellate in the stomach of an insect which ingests suitable pabulum rich in blood. In October 1911 Captain Cragg, I.M.S., kindly gave me a female *Conorrhinus rubrofasciatus* which was caught in his house at night. This bug was fed on myself, and during November, December and January, laid a large number of eggs, many of these were separated and a number of the first nymphal instars were bred out; as this bug is naturally infected with a *crithidia* it is important to carefully exclude it, and this was at once accomplished by breeding out the young stages apart from the parent bug. These nymphs were fed on R. on January 30th and again on 31st, and the first bug was dissected on the 1st; it was found to have ingested about 500 parasites, and they were still lying in the leucocytes which were unchanged. The next was dissected on the 2nd and now it was found that the parasites had degenerated in the leucocytes, and that there was not a trace of any development; in others examined later the parasites had entirely disappeared. The first nymphal instar of this bug is about as large as the adult of *Cimex*, and it ingests about twice as much blood, in fact it is a voracious blood-sucker, becoming so distended as to be almost unrecognisable. Here we have an insect whose midgut when distended with blood closely simulates the culture tube containing rich nutrient fluid, and yet though it may ingest hundreds of parasites they never flagellate but at once degenerate. This observation not only proves that this insect can never carry this parasite in nature but clearly shews that the parasite of Kala Azar will not flagellate in still another blood-sucking insect. As a result of these observations I am justified in concluding that the bed-bugs *Cimex rotundatus* and *Cimex lectularius* are the true invertebrate hosts of the parasite Kala Azar.

The Biological Position of the Parasite.

I have so far not attempted to give a detailed reason for my having previously placed this parasite in the genus *Herpetomonas* Kent, for the reason that up to the present I was unable to study its complete extra-corporeal life history. Now however I am in a position to examine all the evidence in favour of this view. At present these parasites are placed in a most anomalous position as regards their relationships and affinities, for the genus *Leishmania*, Ross, has so far as I am aware never been defined since Ross created it in 1903; for reasons that are obvious, his definition cannot hold to-day. It is not then to be wondered that they are placed by some authorities close to the *Piroplasmata*, and at present all that can be said regarding their nature is that they are

flagellates. I have now shewn that one of these parasites is more than a mere flagellate, and that it is a true insect parasite, in that it undergoes its complete life history in the alimentary tract of an insect, and that human blood ingested by this insect is injurious to its development. This fact strongly suggests that it was originally a harmless parasite of the bed-bug, and that as this insect has slowly become sanguivorous in its habits, the life history of the parasite has thus become modified ; and that it is no longer found in its natural state in the bug. It can however still maintain itself in the bug, but only under very special conditions, that is, when the bug only feeds once on a person infected with Kala Azar. Here we have a remarkable adaptation of a parasite to the altered habits of its host. Are we however justified in considering this to be the probable origin of this human parasite ? In order to answer this question it is necessary to see whether there is at present any known species of insect flagellate whose life history is shewing signs of modification. There is fortunately a good example to be found in the case of the erithidia* (*C. melophagia* Flu.) which is parasitic in *Melophagus ovinus* for Miss Porter who has carefully studied its life history finds that it may be transmitted from one ked to another by means of the post-flagellates passed out in the excreta of the insect, but that this is not now the regular method of the transmission of this flagellate ; it now nearly always passes from one insect to another by hereditary transmission. It is however interesting to know that this parasite may still be transmitted by the simplest means. Another instance is I believe to be found in the herpetomonads of mosquitoes. These flagellates almost invariably undergo their post-flagellate stage in the hind guts of their hosts, but they may occasionally round up in the midgut ; this is a very suggestive observation for it will explain how such a parasite may become transferred to other hosts. It is only recently that the life histories of these insect flagellates have been studied with any degree of precision, and I have little doubt that when such forms occur in blood-sucking insects as for instance *C. conorrhini* in *Conorrhinus rubrofasciatus*, they will enable us to settle the question regarding the origin of the parasite of Kala Azar, for here we have an insect which at the present time is altering its habits and becoming a blood-sucker.

The parasite of Kala Azar is then a true insect flagellate and like all these forms it has three phases in its life history, *pre-flagellate*, *flagellate*, and *post-flagellate* ; in its *pre-flagellate* stage it is a round or oval body which divides by simple longitudinal fission, or by multiple segmentation. In its *flagellate stage* it multiplies by rosette formation and later by longitudinal division, which may

* I am entirely in agreement with Miss Porter and Dr. Swingle that the flagellate found in *M. ovinus* is a true insect parasite, and that it has no connection with any trypanosome which may occur in the blood of a sheep.

be equal or unequal. In its *post-flagellate stage* the flagellates shorten and their flagella are shed, they then divide several times and finally come back to the round or oval stage containing a nucleus and blepharoplast. From a reference to my definition of the genus *Herpetomonas* given in 1908, it will be seen that the parasite of Kala Azar corresponds exactly to it, and I have already shewn that there are several species which are distinguishable from it, I can therefore see no reason for creating a new genus for it. As the parasite of sleeping sickness passes one stage of its life history in the blood and organs of man, and its multiplicative stage in an invertebrate, *Glossina palpalis*, so in the same way the parasite of Indian Kala Azar passes one stage of the life history in the blood and organs of man and its multiplicative stage in an invertebrate, *Cimex rotundatus*; there would however be no justification for placing the parasite of Sleeping Sickness in any other genus but the *Trypanosoma*. By placing the parasite of Kala Azar in the genus *Herpetomonas* its relationships and affinities are exactly defined, and there can be no possible confusion regarding its nature. It should therefore in future be known as *Herpetomonas donovani*, Laveran and Mesnil.

Kala Azar in Madras.

Since Donovan's discovery of the disease in Madras, it is now known to be one of the important causes of mortality; owing however to the fact that it is a chronic disease, it is apt to be lost sight of, and the vast majority of those attacked have no idea whatever that they are victims of this deadly disease. Unfortunately it is impossible to even guess the number of those who annually die of it. The reason for this is simple, it is an insidious disease, developing slowly and taking months before it proves fatal; it is frequently overlooked, and it is commonly diagnosed as typhoid; death may result from exhaustion, dysentery, pneumonia, peritonitis, or from heart failure, the result of advanced emaciation. Death is then stated to be due to one or other of these causes. I have no doubt therefore that the available statistics relating to this disease represent only a small number of those who actually die of it. Recently these sufferers have been disinclined to enter a hospital, for it is now becoming known that very little can be done for them, so that even hospital statistics do not represent the extent of the disease in Madras. It is prevalent in certain streets of George Town, in Royapuram, in Vepery, in Puraswakam, in Choolai, in Triplicane, and I have seen cases from other parts of the Town. It is then widely distributed. In order to find out the real extent of the disease in Madras, and as to whether it is spreading, the co-operation of the people themselves is most desirable; the Indian practitioners who doubtless see most of the cases could render valuable assistance in mapping out the distribution and

extent. Public lectures of a simple nature describing the disease, its parasite and the danger of allowing bugs to feed on a patient, would help in diffusing knowledge regarding this fatal malady. To those who are anxious to avoid it, I have no hesitation in saying that the bed-bug is the transmitter, and great care should be taken to prevent bugs from feeding on a patient with marked dysentery, as such a patient generally has large numbers of parasites in his circulating blood.

The main conclusion to be drawn from these new observations is as follows :— The parasites taken up by a bug, in its last feed from an infected person, are the only ones which can develop, and they can only complete their development, provided the bug does not again feed before the final post-flagellates are formed. This statement may have however to be modified later. I need hardly say that many more experiments will be carried out in order to clear up some points which are yet obscure, for instance, it is important to know whether bugs fed on a case whose blood contains a few parasites, will shew a heavy infection with flagellates; also whether the parasite can complete its development, in any stage of the bug, and during the Madras hot weather temperature. It is hoped also to carry out some immunity experiments in order to see whether a healthy person's blood will destroy the flagellates, and as to whether this will lead to some satisfactory form of treatment. Experiments will also be carried out with animals, the dog for instance, to again see whether it is the natural reservoir of the parasite in Madras. Transmission experiments with both *Cimex rotundatus* and *Cimex lectularius* and the parasite of Oriental Sore are now in progress and from the new facts discovered as a result of the further study of the allied parasite, I have now every hope of transmitting this parasite by the bed-bug.

NOTE.

Since the above memoir was written, I have succeeded in infecting a white rat, by inoculating it intra-peritoneally with 3 c. c. of emulsion, made from a spleen, removed *post mortem* from a patient who died of Kala Azar. The rat died two hours after it was given a second inoculation of 1 c. c. of a similar emulsion; this being 15 days after the first inoculation. Its liver and spleen were considerably enlarged and contained large numbers of the typical parasites. None were found in the bone-marrow. It will be remembered that Laveran and Petit have carried out a very similar experiment with *Herpetomonas infantum* Nicolle, but the white rats they inoculated never showed any parasites in their organs; this in my opinion suggests that the two parasites are totally distinct species. I have also inoculated monkeys, dogs, jackals, rabbits, guinea-pigs, cats, a calf, a pig and a goat, but at present these animals show no signs of having contracted the disease. The above animals, as well as other white rats, similarly inoculated, will be kept under observation for a long time so that there will then be no doubt as to whether they are susceptible or not.

EXPLANATION OF PLATES.

- Figure 1. A large mononuclear shewing the early changes towards disintegration ; it contains a single unchanged parasite. *Cimex lectularius*.
- „ 2. A polymorphonuclear leucocyte also disintegrating ; it contains a single unchanged parasite. *Cimex lectularius*.
- „ 3. A similar cell. *Cimex lectularius*.
- „ 4. Another polymorphonuclear leucocyte. *Cimex lectularius*.
- „ 5. A large mononuclear leucocyte, it contains a parasite which shews the earliest changes towards development. *Cimex lectularius*.
- „ 6. A free parasite also shewing early developmental changes. *Cimex lectularius*.
- „ 7. A much enlarged parasite lying at the margin of a disintegrated leucocyte. These are the forms which it is important to observe in the insect which is suspected to be the probable carrier. *Cimex lectularius*.
- „ 8. A free parasite, the protoplasm stains blue and is granular, the nucleus is enlarged and kidney shaped, and the blepharoplast is thickened and elongated. *Cimex rotundatus*.
- Figures 9, 10 and 11. Free parasites, the same as that depicted in figure 8. *Cimex rotundatus*.
- Figure 12. A still further enlarged parasite, the nucleus has divided, the blepharoplast is elongated, and the parasite itself about to divide. Lying between the blepharoplast and the margin of the cell is the thickened myoneme which is stretched out and shews the several strands. *Cimex rotundatus*.
- „ 13. A similar parasite, the blepharoplast has here however divided before the nucleus. *Cimex rotundatus*.
- „ 14. Another similar form. *Cimex rotundatus*.
- „ 15. Two enlarged parasites which have resulted from the division of a parasite similar to that shewn in figure 14, in one the flagellum is just about to be extruded. *Cimex rotundatus*.
- „ 16. Two young flagellates attached by the whole length of their bodies ; such a pair would originate from the parasite depicted in figure 13. *Cimex rotundatus*.
- „ 17. Another pair of young flagellates attached only by their posterior ends. *Cimex rotundatus*.
- „ 18. A young growing parasite shewing the thickened flagellar myoneme, the appearance of a vacuole is not seen. *Cimex rotundatus*.
- „ 19. Another similar parasite, here however the flagellar myoneme has the appearance of a vacuole with a brush of filaments lying in it. *Cimex rotundatus*.

- Figure 20. Another similar parasite, the flagellum is seen as a single band lying across the vacuole. *Cimex rotundatus*.
- „ 21. Similar flagellate, two flagella are growing out. *Cimex rotundatus*.
- „ 22. A larger form, both flagella have now freed themselves. *Cimex rotundatus*.
- „ 23. A round flagellate, the nucleus has divided, and the blepharoplast is about to divide. *Cimex rotundatus*.
- „ 24. Twin flagellates about to divide further. *Cimex rotundatus*.
- „ 25. A young flagellate dividing. *Cimex rotundatus*.
- „ 26. An elongated flagellate about to divide. *Cimex lectularius*.
- „ 27. Twin flagellates dividing further. *Cimex lectularius*.
- „ 28. Spindle-shaped flagellate shewing multiple segmentation. *Cimex lectularius*.
- „ 29. Multiple segmentation in the flagellate stage. *Cimex rotundatus*.
- „ 30. Young elongated flagellates attached by their poles. *Cimex lectularius*.
- „ 31. A group of three elongated flagellates. *Cimex lectularius*.
- „ 32. Twin flagellates shewing multiple segmentation into six. *Cimex lectularius*.
- „ 33. Large round parasite shewing multiple segmentation into eight. *Cimex lectularius*.
- „ 34. A large spindle-shaped flagellate. *Cimex lectularius*.
- „ 35. The typical adult flagellate stage of *Herpetomonas donovani* *Cimex lectularius*.
- „ 36. Two small flagellates with long flagella. *Cimex lectularius*.
- „ 37. The early stage of multiple segmentation. *Cimex lectularius*.
- Figures 38 and 39. Small flagellates undergoing multiple segmentation. *Cimex lectularius*.
- Figure 40. Three flagellates which were attached to a cell by their flagella. *Cimex lectularius*.
- „ 41. A large round flagellate, its nucleus has divided up, its blepharoplast is still undivided, and it has a single long flagellum. *Cimex lectularius*.
- „ 42. Two long flagellates. *Cimex lectularius*.
- „ 43. Another pair of mature flagellates. *Cimex lectularius*.
- „ 44. A spindle-shaped flagellate shewing a part of its posterior end breaking off. *Cimex lectularius*.
- „ 45. A small rosette. *Cimex lectularius*.
- „ 46. A large rosette containing about twenty-eight parasites. *Cimex lectularius*.
- „ 47. An early post-flagellate, the parasite is rounding up and dividing. *Cimex lectularius*.
- „ 48. Another similar parasite. *Cimex lectularius*.

- Figure 49. Another post-flagellate form, the posterior end is still pointed. *Cimex lectularius*.
- „ 50. A long flagellate about to round up, its flagella have already partly broken off. *Cimex lectularius*.
- Figures 51 to 66. Various small post-flagellates, they shew the light staining protoplasm, granular nuclei, and the remains of flagella, several are dividing again. *Cimex lectularius*.
- „ 67 to 73. The final post-flagellate stage. *Cimex lectularius*.

All the figures were drawn with the aid of a camera lucida, with Leitz 1-16 achromatic objective, a No. 8 compensating eyepiece, except figures 2, 3, 4, 5 and 46, which were drawn with a No. 4 eyepiece. It will be noted that most of the drawings were made from *Cimex lectularius*.

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